22. (Amended) The method of claim 1, wherein said therapy-sensitizing gene activity is *p53* [p53] therapy-sensitizing activity.

23. (New) The method of claim 3, wherein said portion of a therapy-sensitizing gene or said portion of a cDNA is introduced to said tumor cell in aerosolized preparation.

REMARKS

I. The Section 112, 1st Paragraph, Rejection

The Examiner objected to the specification and rejected claims 1-22 under 35 U.S.C. § 112, first paragraph, as failing to provide an enabling disclosure. The objection and rejection are respectfully traversed.

A. A patentee may be his or her own lexicographer.

The Examiner's rejection to the definitions of various terms such as "wild-type therapy-sensitizing gene activity" and "tumor cell" is respectfully traversed.

It is a well-established axiom in patent law that a patentee may be his or her own lexicographer and thus may use terms in a manner consistent, contrary to, or inconsistent with one or more of their ordinary meanings. Hormone Res. Found., Inc. v. Genentech, Inc., 15 USPQ2d 1039 (Fed. Cir. 1990). In this case, applicant coined "wild-type therapy-

sensitizing gene activity" to communicate the invention. According to the specification, it is meant a gene or gene product or portions thereof which are deliverable to a tumor cell.

B. There is credible utility for the claimed invention.

On page 6 of the office action, the Examiner stated that "it is not apparent that the *in vitro* data are extrapolatable to the whole animal *in vivo*." It appears that the Examiner doubts the utility of the claimed invention. The rejection on this ground is respectfully traversed.

According to the Examiner Guidelines For Biotech Applications by the PTO, if the applicant has asserted that the claimed invention is useful for any particular purpose and that assertion would be considered credible by a person of ordinary skill in the art, the Examiner should not impose a rejection for lack of utility. An applicant's assertion of utility creates a presumption of utility that will be sufficient, in most cases, to satisfy the utility requirement. Credibility is to be assessed from the perspective of one of ordinary skill in the art in view of any evidence of record (e.g., data, statements, opinions, references, etc.) that is relevant to the applicant's assertions.

The utility of this invention is to improve the therapeutic effect of cancer therapy of tumor cells lacking wild-type therapy-sensitizing gene activity. There is sufficient evidence to support the credibility of the asserted utility in this application.

This application has shown that wild-type *p53* activity transfected into tumor cells sensitizes the cells to chemotherapy and radiation therapy. Two publications by other labs support the utility of the claimed invention.

In Lentz et al. Proc. Am. Assoc. Cancer Res., vol. 36, March 18, 1995 - March 22, 1995, page 21, abstract no.121 (attached to this response), wild-type *p53* was transfected into HL-60 cells, which normally lack both *p53* mRNA and protein; expression of wt *p53* rendered the HL-60 cells 10-fold more sensitive to growth inhibition by the thymidylate synthase-directed drug FdUrd.

In Fujiwara et al. Cancer Res. 54:2287-2291, May 1, 1994 (attached to this response), recombinant adenovirus-mediated transfer of the wild-type *p53* gene into monolayer cultures or multicellular tumor spheroids of human non-small cell lung cancer cell line H358, which has a homozygous deletion of *p53*, markedly increased the cellular sensitivity of these cells to the chemotherapeutic drug cisplatin. Direct injection of the p53-adenovirus construct into tumors implanted on nude mice, followed by the administration of cisplatin, induced massive apoptotic destruction of the tumors. Fujiwara et al. stated that "[t]hese results support the **clinical application** of a regimen combining gene replacement using replication-deficient wild-type *p53* adenovirus and DNA-damaging drugs for treatment of human cancer."

According to the Examiner Guidelines For Biotech Applications, the applicant does not have to prove that there is a statistically proven correlation between characteristics of an invention and the asserted use, nor does he or she have to provide actual *in vivo* utility in

humans to prove utility. Therefore, the Examiner has not articulated sound reasons why a person of ordinary skill in the art would conclude that it is more likely than not that the asserted utility is not credible. There is no *prima facie* showing of lack of utility.

C. The specification in combination with the prior art teaches how to make and use the invention

On page 8 of the office action, the Examiner stated that "it is not apparent that 'delivering wild-type therapy sensitizing gene and activity to a cancer cell is adequately described by the lone example of p53 to only T98G and T98Gp53 cells . . ." The Examiner also stated on page 5 of the office action that the *in vitro* data in the present application would not be extrapolated to the *in vivo* situation by an artisan "in the absence of a demonstrated ability to control time of expression, amount of expression, ability to terminate expression . . ."

However, a patent need not teach, and preferably omits, what is well known in the art. Hybritech Inc. v. Monoclonal Antibodies, Inc., 231 USPQ 81, 94 (Fed. Cir. 1986). It is not necessary for an applicant to disclose in his application aspects of his invention which are within the general knowledge or skill in the relevant art as of the filing date of the application.

Applicant submits that there are methods known to those skilled in the art to deliver a gene or a protein having wild-type therapy-sensitizing activity to tumor cells. Pages 16-36 of the specification describe nine examples of delivering wild-type therapy-sensitizing

activity to tumor cells, including citations to prior art references describing detailed methodology. The references cited by the Examiner in the section 103 rejection also provide methods for delivering a gene or a protein to tumor cells.

A considerable amount of experimentation is permissible if it is routine. Enablement is not precluded by the necessity for some routine experimentation. In re Wands 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

D. Enablement does not require all possible species to be tested.

The Examiner stated that the claimed invention has not been proven effective against all cancers. It appears that the Examiner would demand the applicant to test all cancers to satisfy the enablement requirement. Such requirement would be against the policy of the patent laws:

> such a requirement would force an inventor seeking adequate patent protection to carry out a prohibitive number of actual experiments. This would tend to discourage inventors from filing patent applications in an unpredictable area since the patent claims would have to be limited to those embodiments which are expressly disclosed.

In re Angstadt and Griffin, 190 USPQ 214, 218 (CCPA, 1976).

For enablement of a generic claim, there is no requirement that every species must be tested. It is not necessary for the enablement of claims 1-22 to test all cancers in the specification as asserted by the Examiner.

In Ex parte Mark, 12 USPQ 1904 (PTO Bd.Pat.App. & Int. 1989), the PTO Board of Patent Appeals and Interferences(the "board") reversed an enablement rejection and held that a claim directed to proteins generally was enabled when the specification only disclosed specific examples. The methods and products in question involved "muteins," described as proteins in which cysteines not essential for biological activity are substituted with another moiety. The Examiner rejected the claims because the specification disclosed only specific examples (IFN-β, IL-2, and TNF) and the claims at issue read on both **operative and inoperative** species. The board reversed. The board pointed to the claim limitation that the mutein had to retain the biological activity of the native protein. Thus inoperative species were, in any event, outside the scope of the claims. The board went on to find that one skilled in the art would be able to routinely determine if such cysteine substitution would be deleterious to biological activity. Thus, the board reasoned, undue experimentation would not be required for one skilled in the art to practice the claimed invention for a given protein:

> The fact that a given protein may not be amenable for use in the present invention in that the cysteine residues are needed for the biological activity of the protein does not militate against a conclusion of enablement. One skilled in the art is clearly enabled to performed such work as needed to determine whether the cysteine residues of a given protein are needed for retention of biological activity.

12 USPQ at 1907. Moreover, with regard to as yet-undiscovered proteins and polypeptides, the board in Mark held that it would be within the skill of the art to test such proteins to see if they retained biological activity after cysteine substitution.

This invention is directed to treating tumor cells lacking wild-type therapy-sensitizing activity. Such tumor cells may be identified by methods known to one skilled in the art (e.g. page 6 - 26 of the specification).

> Experimentation is not inconsistent with enablement, providing that it is not undue. Thus, the fact that experimentation may be complex, as testified to in this case, does not necessarily make it undue, if the art typically engages in such experimentation.

In re Certain Limited-Charge Cell Culture Microcarriers, 221 USPQ 1165, 1174 (USITC 1983).

Given the availability of screening assays in the prior art for identifying mutations in therapy-sensitizing genes, one skilled in the art is clearly enabled to identify tumor cells susceptible to the treatment of this invention.

E. The Examiner has failed to carry the burden of proof.

> [A] specification disclosure which contains a teaching of the manner and process of using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein . . . it is incumbent upon the Patent Office whenever a rejection on this basis is made to explain why it doubts that truth or accuracy of any statement in the supporting disclosure and back up assertions of its own with acceptable evidence or reasoning which is inconsistent with contested statement.

In re Marzocchi, 169 USPQ 367, 369, 370 (C.C.P.A. 1971). However, the Examiner has failed to point out a lack of enablement for any specific claim of this invention or prove total incapacity of the claimed invention. Therefore, applicant submits that the objection and rejection under 35 U.S.C. § 112, first paragraph should be withdrawn.

II. The Section 112, 2nd Paragraph, Rejection

Applicant submits that the amendment to claims 1, 2, 3, 9, 17, 21 and 22 overcomes the rejection to these claims under 35 U.S.C. § 112, second paragraph for being indefinite.

In claim 6, "biological therapy" has a meaning to one skilled in the art. For example, a reference cited by the Examiner in the section 103 rejection (Moosa et al.) described biological therapies.

The Examiner's objection to the use of the term "portion" is respectfully traversed because the specification (e.g. pages 6 and 7) has defined the portion of a wild-type therapy-sensitizing gene or protein having the ability to sensitize a tumor cell to cancer therapy.

III. The Section 103 Rejection

The Examiner rejected claims 1-22 under 35 U.S.C. § 103 as being obvious over the prior art. This rejection is respectfully traversed.

A. Prior art cited by the Examiner

(1) Cheng et al. infected the human T leukemia cell line Be-13, which lacks endogenous p53 protein, with a recombinant retrovirus encoding the wild-type allele of human *p53* gene. Expression of *p53* reduced the growth rate of infected Be-13 cells *in vitro*, suppressed colony formation in methylcellulose cultures, and abrogated Be-13 cells' tumorigenic phenotype in nude mice.

(2) Itoh et al. isolated cDNA encoding human Fas antigen determinant from human T cell lymphoma KT-3 cells. When they expressed the cDNA in murine T cell lymphoma WR19L or fibroblast L929, the transformed cells were killed by mouse anti-Fas antibody by apoptosis.

(3) Malkin et al. described that alterations of the *p53* gene occur not only as somatic mutations in human cancers, but also as germ line mutations in some cancer-prone families.

(4) Nabel et al. described delivering proteins to discrete blood vessel segments by catheterization using genetically modified or normal cells or other vector systems.

(5) Srivastava described hybrid parvovirus vectors for gene therapy, e.g. delivering constitutive levels of a pharmaceutical product or producing a recombinant protein.

(6) Wu et al. described a targetable gene delivery system for introducing foreign genes into mammalian cells utilizing receptor-mediated endocytosis.

(7) Moosa et al. described conventional cancer therapies such as radiation therapy, chemotherapy, biological therapy, cryotherapy and hyperthermia.

The Examiner argued that because Cheng et al. disclosed that expression of the wild-type allele of *p53* suppressed the tumorigenic phenotype of Be-13 cells *in vivo*; Srivastava disclosed vectors that are indicated as safe for gene therapy; and Moosa et al. described radiation therapy, chemotherapy and other treatment methods; it would have been obvious to anyone of ordinary skill in the art to deliver a DNA encoding a tumor sensitizing product to an afflicted individual along with routine known and established therapies.

B. Burden of proof by the Examiner

> The Examiner bears the burden of establishing a *prima facie* case of obviousness. Only if this burden is met does the burden of coming forward with rebuttal argument or evidence shift to the applicant. When the references cited by the Examiner fail to establish a *prima facie* case of obviousness, the rejection is improper and will be overturned. (citations omitted) In re Deuel, 34 USPQ2d 1210, 1214 (Fed. Cir. 1995).

As discussed below, the references cited by the Examiner fail to establish a *prima facie* case of obviousness.

C. The differences between the prior art and the claimed invention.

To determine patentability under § 103, it is necessary to determine the difference between the prior art and the claimed invention, and then determine if the differences are such that the subject matter sought to be patented as a whole would have been obvious to a person of skill in the art at the time the invention was made. The issue is not whether the differences between the prior art and the claimed invention would have been obvious, but whether the subject matter as a whole would have been obvious. Graham v. John Deere Co., 383 U.S. 1, 148 USPQ 459 (1966).

The claimed invention increases the therapeutic effect of a conventional cancer therapy with a novel process that contains at least two steps:

(1) deliver wild-type therapy-sensitizing gene activity - through a protein or a gene or portions thereof - to a tumor cell that has lost its wild-type therapy-sensitizing gene activity; and then

(2) subject the tumor cell to a conventional cancer therapy such as radiation therapy, chemotherapy, biological therapy, cryotherapy or hyperthermia.

When evaluating a claim for determining obviousness, all limitations of the claim must be evaluated. The invention must be reviewed as a whole. In re Gulack, 217 USPQ 401 (Fed. Cir. 1983).

Although Cheng et al. described that the expression of wild-type *p53* reduced the growth rate of tumor cells *in vitro* and *in vivo*, it did not describe or suggest that wild-type *p53* makes the tumor cells more sensitive to any conventional cancer therapy. Moosa et al. described conventional cancer therapies. Wu et al., Srivastava, and Nabel et al. described methods of delivering gene or protein to cells. Malkin et al. described the prevalence of *p53* mutations in various cancer or pre-cancer cells. Itoh et al. described cloning the Fas antigen and an antibody against the Fas antigen.

None of the prior art references cited by the Examiner described that a gene or its encoded protein may be used to make a tumor cell lacking wild-type therapy sensitizing activity more susceptible to a conventional cancer therapy. Neither do they describe or suggest combining the step of delivering wild-type therapy-sensitizing gene activity to the tumor cell with the step of treating the tumor cell with a conventional therapy.

D. No suggestion or motivation to make the claimed invention in the cited references

The claimed subject matter as a whole is not obvious unless a person of skill in the art would have been prompted, in view of the teachings of the prior art, to be led to the claimed invention. Where one element of the claimed invention is found in one reference, and

another element of the claimed invention is found in another reference, the teachings of the two references can be combined **only if** there is some suggestion or incentive to do so. In re Fine, 5 USPQ2d 1596, 1599 (Fed. Cir. 1988). In addition, the motivation or suggestion for combining the teaching must be other than the knowledge learned from the disclosure of the applicant. In re Laskowski, 10 USPQ2d 1397, 1398 (Fed. Cir. 1989). In this case, no suggestion or motivation was given in the references cited by the Examiner.

Not until after the parent application of this CIP application was filed on April 29, 1994 were there publications that described using wild-type *p53* to sensitize tumor cells to conventional cancer drugs.

The only way the disclosures of Cheng et al. and other references cited by the Examiner can be read to result in the above statement is with benefit of applicant's disclosure. Using applicant's description that a wild-type therapy sensitizing gene such as *p53* can make a tumor cell more susceptible to conventional cancer therapies, the Examiner selected references which described parts of the claimed process albeit they did not describe the whole process or suggest combining the parts to make the whole process.

Such use of applicant's disclosure is improper. Selective hindsight cannot be used to evaluate obviousness. There must be a reason or suggestion in the prior art for selecting the procedure used, other than the knowledge learned from the applicant's disclosure. In re Dow Chem. Co., 5 USPQ2d 1529, 1532 (Fed. Cir. 1988).

> A patentable invention may lie in the discovery of the source of a problem even though the remedy may be obvious once the source of the problem is identified. This is part of the "subject matter as a whole" which should always be considered in determining the obviousness of an invention under 35 U.S.C. § 103. In re Peehs, 204 USPQ 835, 837 (CCPA, 1980).

Cancer therapy and tumor suppressor genes such as *p53* have been under intensive study. However, prior art does not render a claimed invention obvious when the prior art references might have piqued a scientist's curiosity to further investigate the role of *p53* in tumor growth, but the prior art itself does not contain a teaching of how to make the invention or how the claimed result would be obtained if certain processes were pursued. The claimed invention is not obvious in view of Cheng et al. and the other cited references because they provided no indication that adding wild-type *p53* or any other therapy sensitizing gene activity to a tumor cell having mutated *p53* activity or other abnormal therapy-sensitizing gene activity would sensitize the tumor cell to chemotherapy. Therefore, it is respectfully submitted that Cheng et al., Moosa et al., Wu et al., Srivastava, Nabel et al., Malkin et al., and Itoh et al. do not provide a *prima facie* case of obviousness.

Accordingly, the claims are now in condition for allowance and a notice to that effect is respectfully requested. If there is any fee due in connection with this response, please charge Deposit Account No. 12-2475 for the appropriate amount.

Respectfully submitted,

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BIOLOGY

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Effect of p53 activity on the cytotoxicity of 5-fluoro-2'-deoxyuridine and its inhibition of thymidylate synthase. Lenz, H.J., Ju, J.-F., Danenberg, K.D., Banerjee, D., Bertino, J.R., and Danenberg, P.V. University of Southern California, Los Angeles, CA 90033, and Memorial Sloan Kettering Cancer Center, New York, NY 10021.

To study the effects of the tumor-suppressor p53 on sensitivity of cells to the thymidylate synthase (TS)-directed drug 5-fluoro-2'-deoxyuridine (FdUrd), wild-type p53 was transfected into HL-60 cells, which normally lack both p53 mRNA and protein because of a major deletion in the p53 gene. Expression of wt p53 rendered the HL-60 cells 10-fold more sensitive to growth inhibition by FdURd. Synchronization of the cells with mimosine before FdUrd treatment caused a further 10-fold increase in the sensitivity of the cells to FdURd. In situ TS assays showed that the growth-inhibitory effect of FdURd was directly proportional to the amount of inhibition of TS activity, but the same TS inhibition was achieved at 10-fold lower levels of FdUrd in the p53 (+) than in the p53 (-) cells. This differential effect of FdUrd on TS activity was not due to p53-induced changes in either TS levels or thymidine kinase activity. It may be associated with the fact that the p53 (+) cells appeared to remain blocked in G1 after administration of FdUrd, while the p53 (-) cells proceeded into S.

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Evidence for regrowth resistance in head and neck tumors(HNT). Preisler HD, Kotelnikov V, Taylor S. Rush Cancer Institute, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612.

Tumor proliferation during treatment is believed offset the effects of cytotoxic therapy thereby contributing to treatment failure, a phenomenon termed regrowth resistance (Preisler, Cell Prolif.1994). We are studying this phenomenon by administering IUdr before and BrdU during treatment the treatment of HNT (chemotherapy or chemo-radiotherapy) to assess cell proliferation. To the time of this abstract we have studied 8 pts with data available for 4. All tumors were responding to treatment. The matched %S phase cells before and during treatment (day 28-40, 1-5 days after the last dose of treatment) were 32 and 22%, 33 and 30%, 29 and 7%, and 2.5 and 3.5%. Labeled tumor cells were either diffusely distributed within the tumor or were restricted to islands of rapidly proliferating cells embedded in fibrotic tissue. Some BrdU labeled cells also contained IUdr indicating that cells which were proliferating before the start of treatment were still proliferating after 1 month of treatment. As can be seen, in 3 tumors the proliferation rates before and during treatment were very similar demonstrating that continued proliferation does occur during treatment thereby offsetting, to some extent, the effects of cytotoxic treatment. These observations strongly suggest that the administration of biological agents to reduce cell proliferation between courses of treatment would improve treatment outcome by reducing regrowth resistance. These studies conotnue and data on at least 8 patients will be presented.

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Dominant Effect of Transduced Wild-type p53 Over Endogenous Mutant p53 in Sensitizing Tumor Cells to Therapy. GIERSET, RA, TURLA, ST; SCALISE, JJ; SOBOL, RE; SHAWLER, DL; HOPKINS, PJ. San Diego Regional Cancer Center, 3099 Science Park Road, San Diego, CA 92121.

We have investigated how introduction of wild-type p53 into glioblastoma cells expressing endogenous mutant p53 affects their sensitivity to chemotherapeutic drugs and radiation. P53 is known to play a key role in triggering apoptosis in normal hematopoietic cells and fibroblasts which have sustained DNA damage, and it could therefore be a factor in the response of cancer cells to DNA damaging drugs and radiation. Following wild-type p53 gene transfer, we selected stable transfectants and characterized them with respect to wild-type p53 gene integration and expression. We found that cells which expressed wild-type p53 had markedly enhanced sensitivity to the chemotherapeutic drug, cisplatin, and to gamma radiation, compared to parental cells, control vector-transduced cells, or transduced cells which have lost expression of wild-type p53. We observed that wild-type p53-expressing cells could be virtually abolished with doses of cisplatin that did not eradicate parental cells or control vector-transduced cells. Enhanced cisplatin sensitivity was associated with enhanced susceptibility to apoptosis. This suggests that p53 gene therapy, even under conditions providing only transient expression, could provide a means to enhance or restore apoptotic pathways and enhance sensitivity to conventional treatments.

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Analysis of altered intracellular doxorubicin distribution in primary human breast adenocarcinomas using confocal microscopy. Sognier, M.A., Wadhwani, S., Altenberg, G.A., Lin, J.T., and Belli, J.A. UT Medical Branch, Galveston, TX 77555-0656

Drug sequestration in intracellular vesicles and/or nuclear drug exclusion have been associated with drug resistance. The prevalence of the drug sequestration phenomenon and its relationship to nuclear drug exclusion in primary human adenocarcinomas from untreated patients was investigated. Following mechanical tissue dissociation, intracellular doxorubicin (DOX) distribution (10 ug/ml-2 hrs) was analyzed using confocal microscopy. A higher mean number of DOX containing vesicles/cell was seen in all tumor (1.5-12) versus normal (0.4) samples. The percentage of cells with vesicles within a sample ranged from 8-99% (mean 38%) in tumors compared to 25% in normal. In 4 samples, cells with vesicles had lower mean nuclear/cytoplasmic ratios (NCR) than those without; however, in 3 others, NCR's were similar (as in normals). These results suggest vesicular DOX sequestration is prevalent in human breast tumors and nuclear drug exclusion/vesicular drug sequestration occur independently but frequently coincide.

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Emergence of cisplatin (cDDP) resistance is not affected by p53. Wielders, C.L.C., Tuyt, L., Benbattol, K., Barton, R., Howell, S.B., Los, G. Cancer Center, Univ. of California, San Diego, La Jolla, CA 92093-0812.

We determined whether p53 affected the rate of development of cDDP resistance using the RKO variants CMV.1 ($p53^{+/+}$), RC-Neo ($p53^{+/+}$), p53.13 ($p53^{mut/+}$) and RC-10 ($p53^{-/-}$). We confirmed that the $p53^{+/+}$ cells arrested in G1 after gamma-radiation but that $p53^{-/-}$ did not. However, neither $p53^{+/+}$, $p53^{mut/+}$ nor $p53^{+/-}$ cells arrested in G1 after cDDP treatment. In line with these observations, we demonstrated that the p53 status did not affect the sensitivity of the RKO variants to cDDP. IC_{50} values were 14 ± 2.8, 11 ± 2.4 and 11.4 ± 3.4 μM for the $p53^{+/+}$ RKO, CMV-1 and RC-NEO variants, 9 ± 1.5 μM for the $p53^{mut/+}$ and 12.3 ± 4 μM for the $p53^{+/-}$ variant. The RKO variants were selected for cDDP resistance by exposure to increasing concentrations of cDDP. No difference in the rate of emergence of cDDP resistance could be detected among the RKO variants. Resistance developed slowly (2 fold after 15 selections). Furthermore, platinum accumulation linearly decreased with increasing drug resistance. After 25 selections, a 4 fold resistance in $p53^{+/+}$, $p53^{mut/+}$ and $p53^{+/-}$ resistant variants was associated with a 2 fold decrease in accumulation. In conclusion, p53 does not play a role in either cDDP sensitivity or development of resistance in these human colon carcinoma cells.

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Methotrexate polyglutamates and thymidylate synthase activity in blasts from children with leukemia as a measure of Methotrexate resistance. Frei, E., Weigand, M. and Wiessler, M. Div. Mol. Toxicol., German Cancer Research Center, 69120 Heidelberg, Germany

Resistance to Methotrexate (MTX) is probably the cause of the high mortality of children at relapse, occurring in one out of four common acute lymphoblastic leukemia (cALL) patients. MTX is taken up by cells and converted to polyglutamates (MTXPG) which persist intracellularly. MTX and especially its polyglutamates inhibit the de novo synthesis of thymidine by blocking thymidylate synthase (TS). In freshly isolated blasts, from blood or bone marrow of 11 patients with cALL, 5 with acute myeloblastic leukemia (AML) and from 5 patients at relapse, ^{3}H-MTX uptake, MTXPG -formation and -persistence in MTX-free medium was analyzed. The interindividual variations in total intracellular MTX, MTXPG -chain length and -persistence is quite large. cALL blasts and AML blasts showed the same range of variation. Blasts from patients at relapse either did not take up MTX at all, or showed the same pattern as cALL blasts sensitive to MTX. MTX uptake and metabolism alone is therefore not a suitable prognostic factor for MTX resistance. In some patient blasts we also determined TS and found very low TS activity in blasts from patients at relapse. A low extent of inhibition of TS by MTX was observed in first studies of AML blasts, with a full recovery after 24h in MTX free medium. One mode of resistance to MTX might thus be a highly efficient thymidine salvage pathway or a low sensitivity of TS to MTXPG with a fast recovery after MTX efflux. TS activity alone or in combination with MTX uptake and metabolism could be a good prognostic factor for MTX resistance.